Enhanced proliferation of primary NSCs and sustained differentiation into precursors using heat-stable bFGF

Introduction

Primary neural stem cell (NSC) culture is important for improving the overall understanding of the nervous system and for the advancement of treatments for incurable brain diseases and injuries. These multipotent stem cells are capable of self-renewal and can differentiate into neurons, astrocytes, and oligodendrocytes. *In vitro* methodologies designed to isolate, expand, and functionally characterize NSC populations have revolutionized our understanding of NSC biology. However, isolation and culture of primary NSCs is challenging. This is, in part, due to the dependency on growth factors that are unstable *in vitro*, such as basic fibroblast growth factor (bFGF). The bFGF protein rapidly degrades at 37°C, with a half-life of approximately 8 hours. This contributes to inconsistent levels of active bFGF, which may result in reduced growth rates and spontaneous differentiation of NSCs, even with daily bFGF supplementation. Gibco™ Heat Stable Recombinant Human bFGF Protein (HS bFGF, Cat. No. PHG0360) offers a solution to this problem. Cells respond to HS bFGF just as they would to the native protein. However, whereas native bFGF degrades over time, HS bFGF is engineered to maintain activity at 37°C. Here we demonstrate the ability of HS bFGF to support multipotent primary NSC expansion with minimal spontaneous differentiation and without the need for daily bFGF addition to the culture. We also show that the stability of HS bFGF does not impact downstream differentiation of NSCs, even after multiple passages.

Day	10 ng/mL HS bFGF, every 48 hr	10 ng/mL native bFGF, daily (positive control)	10 ng/mL native bFGF, every 48 hr (negative control)
1	Dissociate tissue and plate cells	Dissociate tissue and plate cells	Dissociate tissue and plate cells
2		Supplement with native bFGF	
3	Complete medium change and HS bFGF supplementation	Complete medium change and native bFGF supplementation	Complete medium change and native bFGF supplementation
4		Supplement with native bFGF	
5	Complete medium change and HS bFGF supplementation	Complete medium change and native bFGF supplementation	Complete medium change and native bFGF supplementation
6		Supplement with native bFGF	
7	Complete medium change and HS bFGF supplementation	Complete medium change and native bFGF supplementation	Complete medium change and native bFGF supplementation
8		Supplement with native bFGF	
9	Passage cells	Passage cells	Passage cells

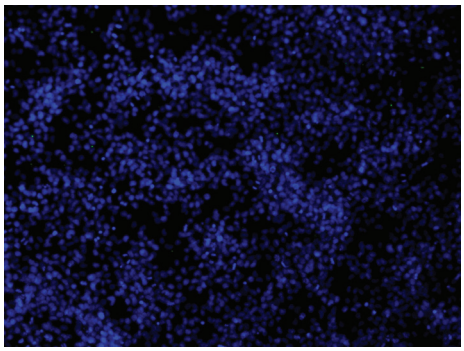
Figure 1. Workflow and media supplementation schedules for NSC expansion.

Experimental workflow

Primary NSCs were isolated from the cortex of E14 Sprague-Dawley rat embryos by dissociation. NSCs were cultured using Gibco™ DMEM/F-12 with GlutaMAX™ Supplement, Gibco™ N-2 Supplement, 1X NEAA, 55 mM β-mercaptoethanol, and 10 ng/mL HS bFGF for 3 passages (Figure 1). As positive and negative control treatments, the cultures were supplemented daily or every other day (every other day being equivalent to the HS bFGF workflow), respectively, with native bFGF at a final concentration of 10 ng/mL. Cells were seeded at 2.6 x 10^4/cm^2 in poly-D-lysine–coated Thermo Scientific™ Nunc™ 6-well plates. The medium was changed every 48 hours and, once the cells reached 85% confluency, the cells were passaged using Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent. Cells were counted on the Invitrogen™ Countess™ II Automated Cell Counter, and the cell counts were then used to calculate and compare doubling times between experimental conditions. We examined maintenance of multipotentcy using MAP2 immunocytochemistry (ICC) to identify spontaneous neuron differentiation (Figure 2). After 3 passages, the cells were cultured for 3 days without HS bFGF or native bFGF, after which we demonstrated trilineage potential by performing ICC using the markers MAP2 (neuron), GFAP (astrocyte), and GALC (oligodendrocyte).

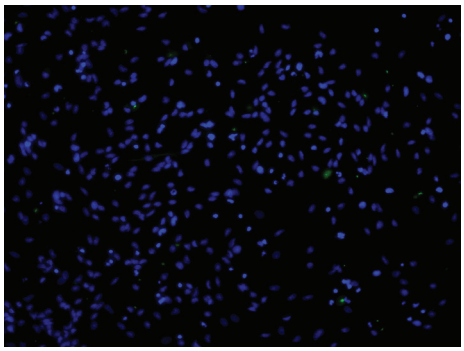
A

HS bFGF every 48 hr



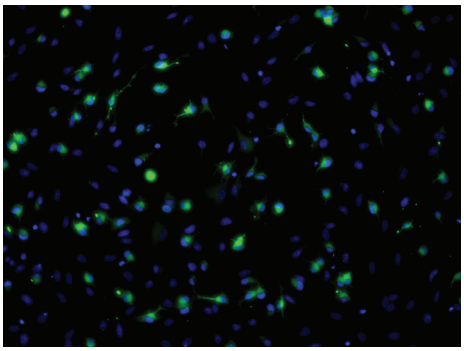
B

Native bFGF every 24 hr



C

Native bFGF every 48 hr



D

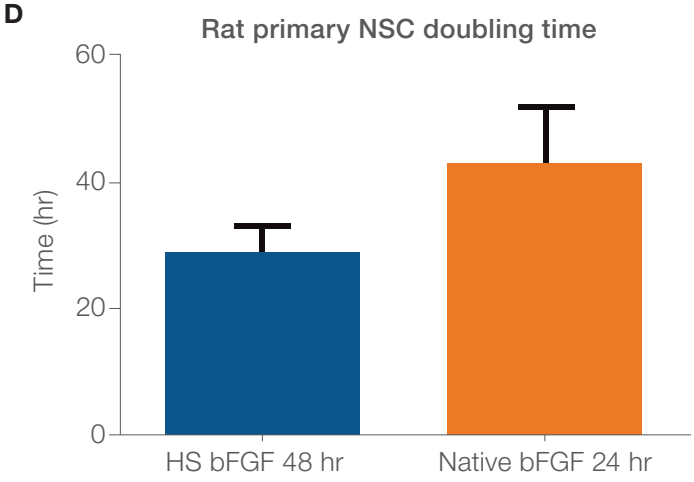


Figure 2. HS bFGF enhances expansion of multipotent NSCs, even without daily feeds. Rat primary NSCs cultured in HS bFGF maintain multipotency **(A)**, similar to when they are supplemented daily with native bFGF **(B)**; both cultures lack MAP2-positive cells (green). Native bFGF does not support multipotency of NSCs when employed using the same supplementation schedule (every 48 hours) that HS bFGF allows **(C)**. Population doubling time of primary NSCs decreases when they are cultured with HS bFGF, without the need for daily supplementation. Average doubling time is based on cell counts at each passage **(D)**.

Results

We found that HS bFGF not only supports the healthy growth and multipotency of primary NSCs using a 48-hour feeding schedule but also results in an increase in growth rate (i.e., shorter doubling time; Figure 2). NSCs cultured with native bFGF using the same workflow as HS bFGF showed spontaneous differentiation starting at passage 1 and increased doubling time with every passage, resulting in complete loss of the NSC culture by the end of passage 3, due to differentiation of cells. NSCs cultured in the medium with daily supplementation with native bFGF or HS bFGF showed equivalent spontaneous trilineage differentiation after 3 days, with both conditions giving rise to neurons, astrocytes, and oligodendrocytes (Figure 3).

Conclusions

Heat Stable Recombinant Human bFGF offers a more flexible workflow for the culture of rat primary NSCs, eliminating bFGF degradation and the need for daily bFGF supplementation. HS bFGF enhanced expansion and preserved multipotency without affecting undirected downstream differentiation into neurons, astrocytes, and oligodendrocytes.

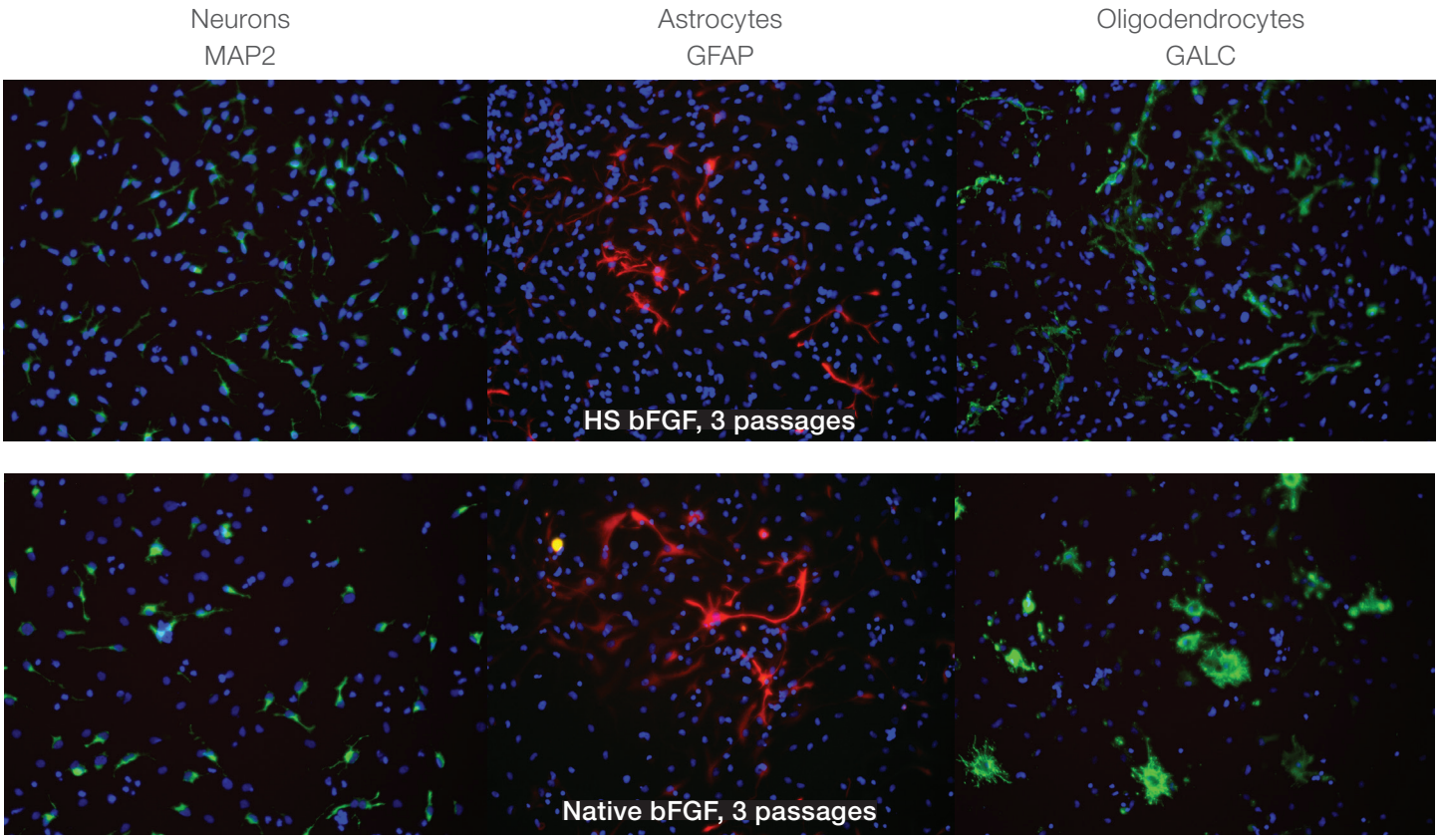


Figure 3. HS bFGF does not interfere with undirected differentiation. NSCs cultured for 3 passages in medium supplemented with HS bFGF every 48 hours or with native bFGF daily showed equivalent ability to differentiate into neurons, astrocytes, or oligodendrocytes upon removal of bFGF, as measured by expression of MAP2 (neuron lineage), GFAP (astrocyte lineage), and GALC (oligodendrocyte lineage).

gibco

Ordering information

Product	Cat. No.
Heat Stable Recombinant Human bFGF Protein, 5 µg	PHG0367
Heat Stable Recombinant Human bFGF Protein, 50 µg	PHG0368
Heat Stable Recombinant Human bFGF Protein, 100 µg	PHG0369
Heat Stable Recombinant Human bFGF Protein, 500 µg	PHG0360
DMEM/F-12, GlutaMAX Supplement	10565018
N-2 Supplement	17502048
Distilled Water	15230162
StemPro Accutase Cell Dissociation Reagent	A1110501
Poly-D-Lysine	A3890401
Nunc Cell-Culture Treated Multidishes	142485
GFAP Monoclonal Antibody (2.2B10)	13-0300
MAP2 Polyclonal Antibody	PA5-17646
GALC Polyclonal Antibody	PA5-76472
Countess II Automated Cell Counter	AMQAX1000
EVOS FL Auto 2 Imaging System	AMAFD2000

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